Using Zero-Mode Waveguides to Unlock Single-Molecule Enzyme Kinetics

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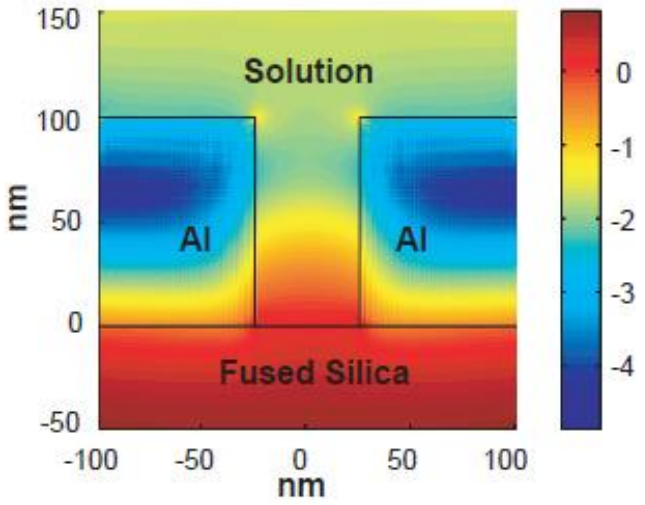
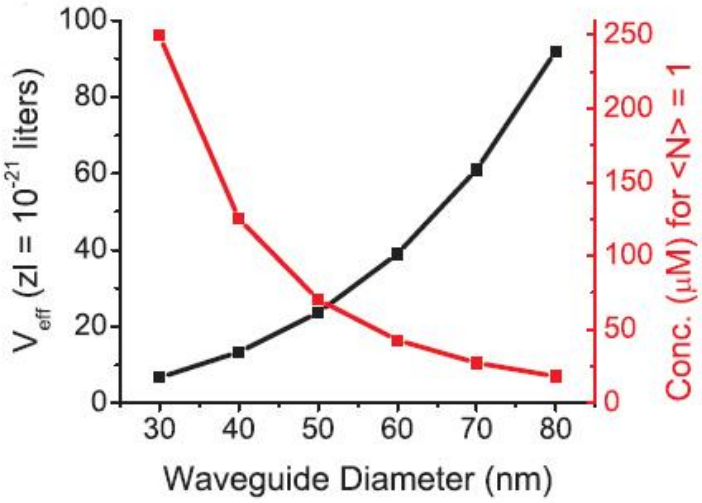
Zero-Mode Waveguide Theory

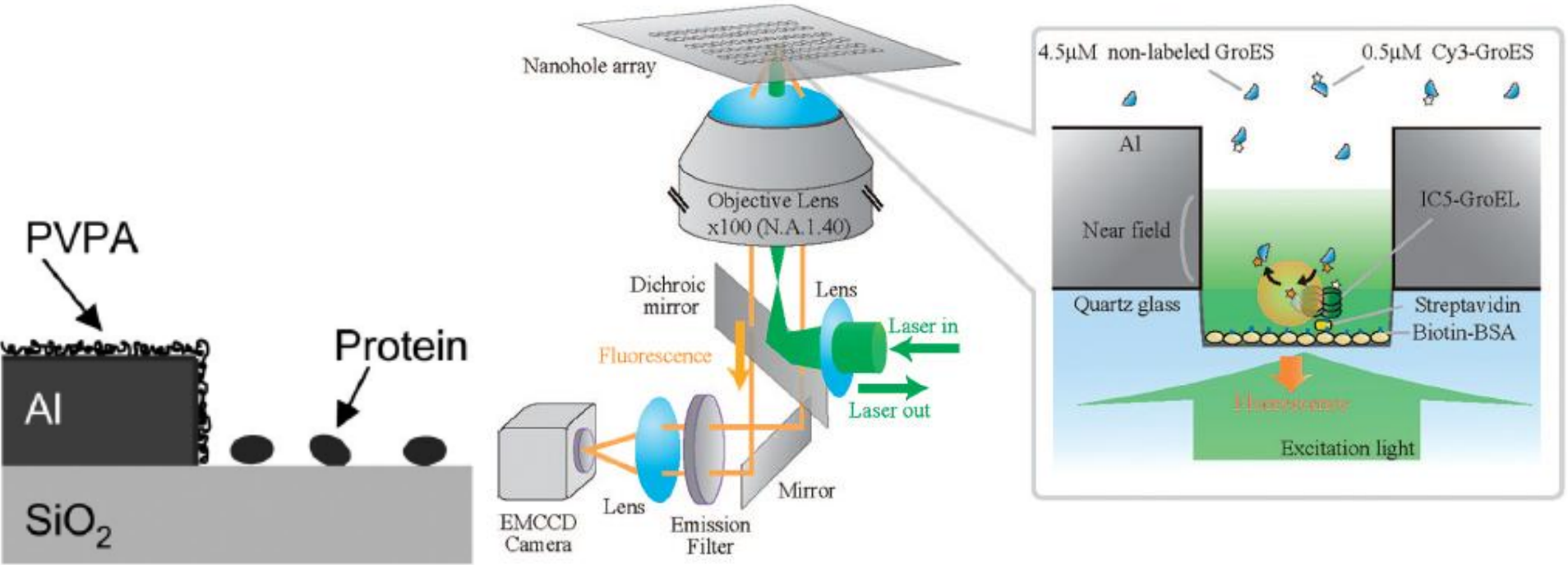
- Metal clad waveguides exhibit cut-off wavelength (λ_c). Above λ_c, waves are evanescent
- Rapid decay of illumination provides a small observation volume
- Existing techniques have a larger observation volume and are limited to low concentration [S] studies.
- ZMW reduces background noise





Zero-Mode Waveguide Application

- ZMW is fused silica substrate coated with thermally evaporated Al film and patterned by lithography
- Specific immobilization of enzyme achieved by PVPA coating
- Existing applications include DNA sequencing, binding kinetics, cell membrane experiments



Single-Molecule Enzymatic kinetics

- Classic Michaelis-Menten equation

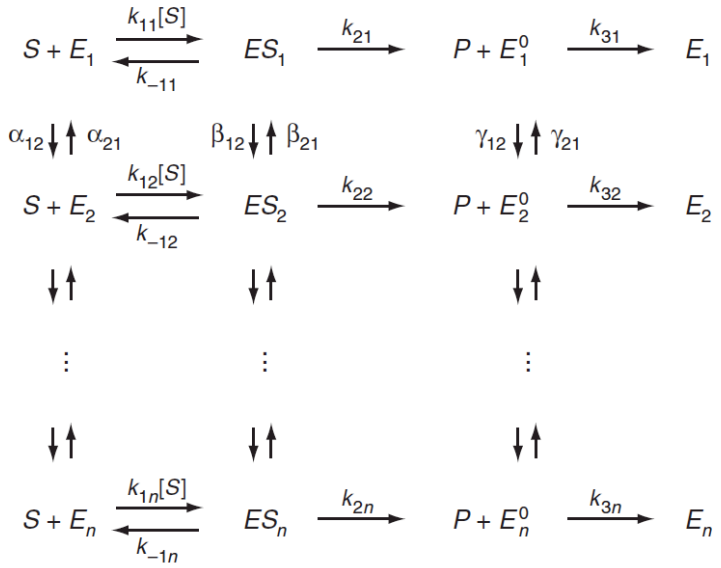
$$\frac{d[S]}{dt} = v = \frac{v_{\max}[S]}{[S]+K_M}$$

- Single-molecule MM equation

$$\frac{1}{\langle\tau\rangle} = \frac{k_2[S]}{[S]+K_M} = \frac{v}{[E]_T}$$

where τ is the time between turnovers

- As [*S*] increases, the reality looks more like scheme 1 where each enzyme conformation has its own kinetic properties

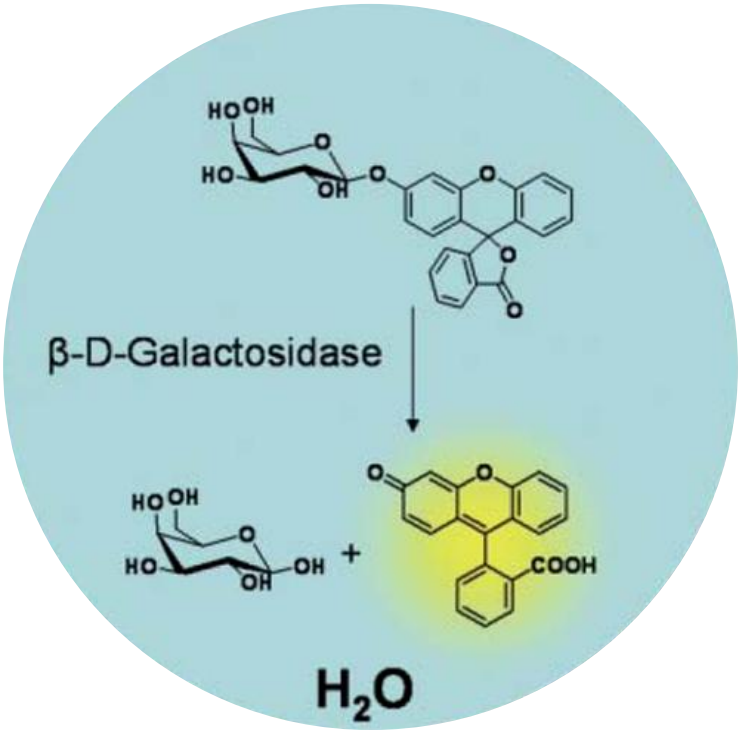


Scheme 1

- Therefore k_2 must be a weight average over all conformations that takes into account the average of k_2 as well as its distribution

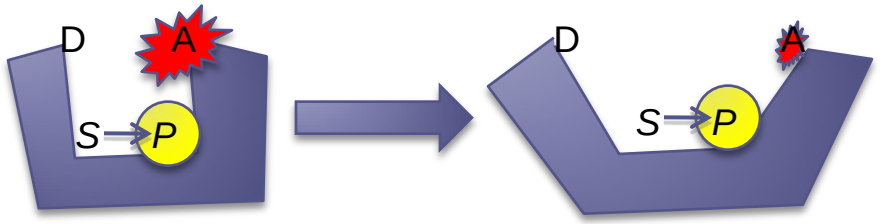
Single Molecule Results

- Fluctuations in turnover rates is highly correlated
  - A quick reaction turnover will probably be followed by another quick reaction turnover
- Some conformations are significantly more active than other conformations
  - The most active conformation can have 1 to 2 orders of magnitude more turnovers than the least active conformation
- Some enzymes only spend 3% of their time in the highly active form
- Opportunity to understand enzymes and bioengineer more efficient enzymes



Proposed study

- Use the ZMW to study kinetics at physiologically relevant substrate concentrations
- Use single molecule FRET to determine what conformations have high or low activity
- Design a molecule that spends more time in its high activity conformation
- β-D-Galactosidase is an ideal enzyme to work with because it is well characterized
- This procedure could be used to increase the activity of any enzyme
- For example, RuBisCO catalyzes CO_2 fixation in all plants. The enzyme is notoriously slow with only ~3 turnovers / second. Bioengineering a more active RuBisCO could reduce greenhouse gases in the atmosphere.



Questions?